REMARKS

Claims 32-41 and 54-63 are pending. Claims 54-63 are new. As explained in more detail below, the new claims find support at pages 4, 6, and 12 of the specification. The examiner has indicated that if priority under 35 USC 119 is desired, the applicants must submit a translation of the foreign application. Applicants note that the present application is a national phase entry from a PCT application, thus priority is under 35 U.S.C. 371. As such, it is not believed that a foreign translation is necessary to establish priority to the German parent application. In order to clarify the claims, the applicants have also amended claim 41 in response to the examiner's objection. The following rejections are at issue and are set forth by number in the order in which they are addressed:

1. Claim 41 is rejected under 35 U.S.C. §112, first paragraph, as allegedly lacking enablement;
2. Claims 32-35, 39 and 40 are rejected under 35 U.S.C. §112, first paragraph, as allegedly lacking an adequate written description;
3. Claims 32, 33, 35, 36, 39 and 40 are rejected under 35 U.S.C. §102(b) as allegedly being anticipated by Zernicka-Goetz et al;
4. Claims 32, 33 and 35-40 are rejected under 35 U.S.C. §103(a) as allegedly being obvious over Zernicka-Goetz et al. in view of Ikawa et al. and in further view of Wobus et al., Sartorelli et al., and Chen et al. and Claims 32-40 are rejected under 35 U.S.C. §103(a) as allegedly being obvious over Zernicka-Goetz et al., Ikawa et al., Wobus et al., Sartorelli et al., and Chen et al. in view of Maltsev and Rohwedel.

1. The claims are enabled

Claim 41 is rejected under 35 U.S.C. §112, first paragraph, as allegedly lacking enablement. The Examiner has requested verification that the deposit has been made under the Budapest Treaty. The Examiner's attention is respectfully directed to Appendix A where the undersigned attorney provides verification the deposit was made in accordance with the Budapest Treaty.

2. **The claims are supported by an adequate written description**

Claims 32-35, 39 and 40 are rejected under 35 U.S.C. §112, first paragraph, as allegedly lacking an adequate written description. The Examiner alleges Applicant did not point to the specification for support for the claim limitation that the promoter is "substantially inactive in undifferentiated embryonic stem cells." Applicant respectfully disagrees and refers the Examiner to page 6 of the previously filed Preliminary Amendment where the applicants stated: "Support for this amendment is found both inherently in the application and at page 4, last paragraph to page 5, second paragraph, page 6, last paragraph, page 12, first paragraph, and page 13, second paragraph." In particular, the specification teaches the following:

i. "This construct is integrated in the native DNA. After the specific activation of intracellular signals, the promoter is activated and the fluorescent protein expressed. Thus, ES cells which activate a cell-specific transcription factor at a certain point of differentiation could be recognized by their fluorescence emissions when being under fluorescent excitation." P. 4.

ii. "Since the ES cells expressing the fluorescent protein become visible in the EB upon activation of heart-specific promoters, the growth of the heart-related cells can be determined under pharmacological/toxicological conditions by a quite simple method." P. 6.

iii. "In contrast to the above described patterns of GFP expression under the control of the beta-actin promoter, ES cells bearing the pCX-(alpha-act)GFP-Neo integrated in the genome exhibit no or only a very weak signal which is visible by fluorescence microscopy. The FACS analysis shows that the average level of green fluorescence of D3 cells containing pCX-(alpha-act)GFP-Neo linearized by SacI is about 35-40 times lower than for cells bearing pCX-(beta-act)GFP-Neo. During the development of the EBs, a right hand shift and enlargement of the initial peak of the GFP fluorescence histogram obtained by FACS was seen." P.

12.

These passages show that that the claimed embodiment was in the possession of the inventors and that the claimed embodiment was specifically contemplated. In particular, the passages specifically describe promoters that are substantially inactive in undifferentiated stem cells. For example, promoters that are substantially inactive can provide average levels of fluorescence that are 35-40 lower than for cells with an active promoter. Applicants further note that the law does require actual literal support for claims terms, only that the claimed embodiments are described. Indeed, the subject matter of a claim need not be described literally or "in ipsis verbis" in order for the specification to satisfy the written description requirement. *See, e.g., In re Lukach*, 169 U.S.P.Q. 795, 796 (CCPA 1971). Furthermore, it is sufficient that the specification convey "convey clearly to those skilled in the art the information that the applicant has invented the specific subject later claimed." *See, e.g., In re Wertheim*, 191 U.S.P.Q. 90, 96 (CCPA 1976); *In re Ruschig*, 154 U.S.P.Q. 118, 123 (CCPA 1967). The passages cited above clearly demonstrate that Applicant invented the claimed invention, including the use of promoters that are substantially inactive in undifferentiated stem cells. As such, Applicants respectfully request that the written description rejection be removed.

Applicants further direct the Examiner to the limitation in newly added claim 54 that promoters in question are "activated after differentiation of the stem cells." The passages provided above clearly describe such promoters.

3. The claims are novel

Claims 32, 33, 35, 36, 39 and 40 are rejected under 35 U.S.C. §102(b) as allegedly being anticipated by Zernicka-Goetz et al. Applicants are puzzled by this rejection as the Examiner has merely repeated arguments relating to (and specifically refers to) canceled claims 15-18, 20, 22-24, and 26-28 and has not given weight to the claim limitation that the promoter is "substantially inactive in undifferentiated stem cells." Applicants respectfully submit that Zernicka-Goetz et al. does not teach the use of such promoters. Specifically, Zernicka-Goetz et al. teach the use of the cdc2 promoter, which is active in dividing, undifferentiated stem cells. See, for example, Zernicka-Goetz

et al. at page 1135, column 2, which teaches that the CDC2 promoter is active in proliferating, undifferentiated cells, and inactive in ES cells that "exit the cell cycle and differentiate in culture." See Hescheler Declaration, presented with the previously filed Preliminary Amendment, Paragraph 3. The cdc2 promoter labels cells from the outset. Thus, tissue-specific expression is not demonstrated. As a result, the claims are not anticipated by Zernicka-Goetz et al.

4. The claims are not obvious

Claims 32, 33 and 35-40 are rejected under 35 U.S.C. §103(a) as allegedly being obvious over Zernicka-Goetz et al. in view of Ikawa et al. and in further view of Wobus et al., Sartorelli et al., and Chen et al. and Claims 32-40 are rejected under 35 U.S.C. §103(a) as allegedly being obvious over Zernicka-Goetz et al., Ikawa et al., Wobus et al., Sartorelli et al., and Chen et al. in view of Maltsev and Rohwedel.

A *prima facie* case of obviousness requires the Examiner to provide a reference(s) which (a) discloses all of the elements of the claimed invention, (b) suggests or motivates one skilled in the art to combine the claimed elements to produce the claimed combination, and (c) provides a reasonable expectation of success should the claimed combination be carried out. Failure to establish any one of these three requirements precludes a finding of a *prima facie* case of obviousness and without more entitles the Applicants to allowance of the claims in issue. *See, e.g., Northern Telecom Inc. v. Datapoint Corp.*, 15 USPQ2d 1321, 1323 (Fed. Cir. 1990). In addressing this rejection, Applicants focus on the independent claims since the non-obviousness of independent claims necessarily leads to the non-obviousness of claims dependent thereon.[1] As explained below, the Examiner cannot establish a prima facie case of obviousness from the previously cited references because there is no motivation to combine the references and no reasonable expectation of success. Furthermore, the examiner has completely failed to address the arguments presented in Applicants previously filed Preliminary Amendment.

a. The Examiner has failed to address Applicant's previous arguments and has failed to consider factual evidence offered by the Applicants

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1 §MPEP 2143.03.

In the present Office Action, the Examiner has essentially repeated his arguments from the last office action in the parent application. This is in spite of the fact that the presently pending claims contain different limitations and the fact that Applicants submitted a fact-based Declaration from Dr. Jurgen Hescheler.

The fact that the Examiner did not consider the new limitations is confirmed by the Examiner's statement at page 8 of the Office Action that "Zernicka-Goetz et al. clearly anticipate claims 32-36, 39 and 40 ... ." This statement is clearly made without consideration of the limitation that the promoter is substantially inactive in undifferentiated embryonic stem cells as described above in Section 3. The fact that the Examiner did not consider the Hescheler Declaration is confirmed because nowhere in the Office Action does the Examiner even mention the Declaration or the facts contained therein.

Such disregard of arguments and factual evidence presented by an Applicant is inexplicable in light of established Federal Circuit precedent, and indeed, the MPEP. The Examiner must respond to all of the arguments and evidence presented by Applicants. The MPEP provides that:

> **Office personnel should consider all rebuttal arguments and evidence presented by applicants**. . . . *In re Beattie*, 974 F.2d 1309, 1313, 24 USPQ2d 1040, 1042-43 (Fed. Cir. 1992). . . . **Office personnel should avoid giving evidence no weight**, except in rare circumstances. *Id. See also In re Alton*, 76 F.3d 1168, 1174-75, 37 USPQ2d 1578, 1582-83 (Fed. Cir. 1996).
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> A determination under 35 U.S.C. 103 should rest on **all the evidence** and should not be influenced by any earlier conclusion. *See, e.g., Piasecki*, 745 F.2d at 1472-73, 223 USPQ at 788; *In re Eli Lilly & Co.*, 902 F.2d 943, 945, 14 USPQ2d 1741, 1743 (Fed. Cir. 1990). Thus, once the applicant has presented rebuttal evidence, Office personnel should **reconsider** any initial obviousness determination in view of the entire record. *See, e.g., Piasecki*, 745 F.2d at 1472, 223 USPQ at 788; *Eli Lilly*, 902 F.2d at 945, 14 USPQ2d at 1743.[2]

Additionally, the Courts have held as follows:

> When *prima facie* obviousness is established and evidence is submitted in rebuttal, the decision-maker must start over . . . . An earlier decision should not . .

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[2] MPEP §§2144.08; emphasis added).

> . be considered as set in concrete, and applicant's rebuttal evidence then be evaluated only its knockdown ability. Analytical fixation on an earlier decision can tend to provide the decision with an undeservedly broadened umbrella effect. *Prima facie* obviousness is a legal conclusion, not a fact. Facts established by rebuttal evidence must be evaluated along with the facts on which the earlier conclusion was reached, not against the conclusion itself. Though the tribunal must begin anew, a final finding of obviousness may of course be reached, but such finding will rest upon evaluation of all facts in evidence, uninfluenced by any earlier conclusion reached . . . upon a different record.[3]

Furthermore:

> If a *prima facie* case is made in the first instance, and if the applicant comes forward with a reasonable rebuttal, whether buttressed by experiment, prior art references, or argument, the entire merits of the matter are to be reweighed. (Emphasis added)[4]

Accordingly, even if the Examiner had established anticipation or a *prima facie* case of obviousness (and Applicant contends that the examiner did not), the Examiner must respond to the information presented in the Declaration. The above directions of the court and the PTO state that the evidence **must be considered**. **The directions specifically state that arguments buttressed by references, such as those contained in the Declaration, must be considered.** Indeed, the Examiner must start over and reconsider the entire anticipation or obviousness analysis.

In the present case, there was no reweighing of the merits by the Examiner. Instead of actually analyzing the arguments relating motivation to combine and the Declaration and the factual evidence contained within it, the Examiner has summarily dismissed the evidence. **This is reversible error.**

As such, Applicants have no choice but to essentially present the arguments made in the previously filed Preliminary Amendment again.

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3 *In re Rinehart*, 531 F.2d 1048, 1052, 189 USPQ 143, 147 (CCPA 1976).

4 *In re Hedges*, 783 F.2d 1038, 1039, 228 USPQ 685, 686 (Fed. Cir. 1986).

b. There is no motivation to combine the references

As the Federal Circuit has stated, "[b]efore the PTO may combine the disclosures of two or more prior art references in order to establish *prima facie* obviousness, there must be some suggestion for doing so, found either in the references themselves or in the knowledge generally available to one of ordinary skill in the art." *In re Jones,* 958 F.2d 347, 351 (Fed. Cir. 1992) (*citing In re Fine,* 837 F.2d 1071, 1074, 5 U.S.P.Q.2d 1596, 1598-99 (Fed. Cir. 1988)); *see also* M.P.E.P. § 2143.01. Instead of relying on objective evidence of record, the Examiner has made the following conclusory motivation statements:

> One having ordinary skill in the art would have been motivated to substitute the beta-actin or CD4 promoter in the pCX-GFP vector used by Ikawa et al. with the promoters described by Wobus et al., Sartorelli et al., and Chen et al. to more easily study the regulatory sequences of the promoter in developmental, pharmacological, and toxicological studies of cardiomyocytes derived from ES cell lines. Office Action, p. 9.
>
> One having ordinary skill in the art would have been motivated to substitute the pCX-GFP vector used by Ikawa et al. with the promoters described by Wobus et al., Sartorelli et al., and Chen et al. to more easily study the regulatory sequences of the promoter in developmental, pharmacological, and toxicological studies of cardiomyocytes derived from ES cell lines. Office Action, p. 9.

These statements are conclusory because they merely recite the references and say the combination would be made to "more easily study" the regulatory sequences. This reasoning is clearly based on hindsight because none of the references themselves mention that a non-cell damaging fluorescent protein could be used or would be useful with the specified promoters. It took the Applicant to make this leap forward. The Federal Circuit has expressly forbidden this approach hindsight-based approach.

> Specifically, the Federal Circuit held that:
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> The factual inquiry whether to combine references must be thorough and searching. It must be based on **objective evidence** of record. **This precedent has been reinforced in myriad decisions, and cannot be dispensed with**.

See, In re Lee, 277 F.3d 1338, 1344 (Fed. Cir. 2002); internal citations omitted; emphasis added. Indeed, the Federal Circuit has made it clear that "[b]road, **conclusory** statements

regarding the teachings of multiple references, standing alone, are not 'evidence.'" *In re Dembiczak*, 175 F.3d 994, 50 USPQ2d 1614 (Fed. Cir. 1999)(emphasis added).

In particular, the Examiner has pointed to nothing in the references themselves or in other prior art that indicates that the vector of Ikawa would be useful with the promoters of Wobus, Sartoelli or Chen. Maltsev and Rohwedel do nothing to cure this deficiency as they are directed to culture techniques. There is simply no objective evidence of record which supports the combination. Importantly, the Examiner has failed to provide evidence of a motivation to utilize a reporter gene encoding a fluorescent protein in conjunction with a promoter that is not substantially expressed in an undifferentiated stem cell.

Finally, Applicant specifically directs the examiner to paragraph 5 of the Hescheler Declaration, which is reproduced below for the Examiner's convenience:

> With respect to Ikawa et al., I note that they describe the use of GFP under the control of the ubiquitously expressed chicken beta-actin promoter. This work has little relevance to the present invention which utilizes development dependent promoters that are substantially inactive in undifferentiated embryonic stem cells to report gene expression in embryoid bodies. It was never envisaged by Ikawa et al. that their GFP system, or something similar to it, could be used in embryonic stem cells. In this respect, I attach as Tab B to this Declaration a printout of an e-mail of 1996 from Dr. Masaru Okabe, head of the Research Institute for Microbial Diseases and senior author of the Ikawa et al. publication, to Drs. Bernd Fleischmann and Eugene Kolossov, who are my co-workers. In his e-mail, Dr. Okabe indicated that h-x-GFP and pCX-GFP are "basically the same to pCX-h-x-GFP" stating that "Our construct may not work nicely in ES cells from our experience. In order to check the high expression of h-x-GFP you had better use other cell lines." Hence, even the authors of Ikawa et al., i.e. those who knew best about GFP in mammalian cells, were of the opinion that GFP is not a suitable marker for ES cells. For the information of the Examiner, I would like to add that the abbreviation h-x was used by Dr. Okabe for EGFP (enhanced green fluorescent protein), which was not published at that time.

As can be seen, the senior author of the Ikawa paper stated that their construct "may not work nicely in ES cells from our experience." Thus, persons skilled in the art with actual experience did not believe their vector would be useful in stem cells. This is factual evidence proving that one skilled in the art would not be motivated to combine the Ikawa vector with the promoter references.

Accordingly, Applicants respectfully submit that the Examiner has failed to establish a *prima facie* case of obviousness because the Federal Circuit standards for motivation to combine have not been met. As such, the obviousness rejection should be removed.

c. There is no motivation to combine the references

The cited references do not provide a reasonable expectation of success for obtaining the claimed methods. The Federal Circuit has held that "obvious to experiment" is not the standard for obviousness. *In re Dow Chemical*, 5 USPQ2d 1529, at 1532 (Fed. Cir. 1988). The Dow court made it very clear that one must determine whether "the prior art would have suggested to one of ordinary skill in the art that this process **should** be carried out and **would** have a reasonable likelihood of success, viewed in light of the prior art." *Id.* at 1531 (Emphasis added).

For the following reasons, Applicants submit that one skilled in the art would not believe that a reasonable expectation of success existed for arriving at the claimed invention.

First, Applicants were the first to show that a fluorescent protein (e.g., GFP) could be expressed in high enough amounts from a development dependent that is not expressed in undifferentiated stem cells to overcome background autofluoresence. As documented Paragraph 5 of the accompanying Declaration by the Inventor, Dr. Jürgen Hescheler, the contemporaneous article Raz et al., Developmental Biology 203:290-294 (1998)(attached as Tab A to the Declaration) teaches that the number of GFP molecules needed to overcome background autofluoresence is relatively high because GFP is noncatalytic. It is estimated that approximately 10^5-10^6 GFP molecules per cell are required to visualize the protein when it is evenly distributed in the cytosol. This low level of sensitivity is expected to result in a time delay between onset of gene expression and the ability to detect accumulated GFP, especially when weak and tightly regulated promoters are used. (See the paragraph bridging the left and right columns of p. 290 of Raz et al.). Thus, a person of skill in the art would not reasonably expect that simply linking a GFP gene to a development dependent promoter would result in detectable expression. Empirical experimentation was required. Moreover, one of ordinary skill in

the art would have had no reasonable expectation of success because prior to the presently claimed invention, it was not clear whether promoter leakiness would cause an unspecific expression pattern or whether time-dependent activation of relevant genes would follow the same pattern in stably transfected ES cells as in untransfected ES cell lines.

In this regard, Applicant specifically directs the Examiner to Example 2 of the present application at page 12, paragraph d). This example shows that when using a vector construct as described in the prior art, i.e. as described in Ikawa et al., together with a cell-specific promoter, such as described in Wobus et al., the person skilled in the art would have encountered problems with background fluorescence because of the presence of the CMV-IE enhancer. Thus, if the person skilled in the art would have tried, as alleged by the Examiner, to combine the vector construct described in Ikawa et al. with any one of the cell- or tissue-specific promoters described in Wobus et al., Sartorelli et al. or Chen et al., it would always have resulted in considerable background fluorescence because the vector construct of Ikawa et al. contains said CMV-IE enhancer. It was only after the present inventor modified the GFP reporter construct that it could be shown that cell- and tissue-specific expression of GFP is possible with a background in undifferentiated cells that compares to non-transfected stem cells.

Second, the cited references in which GFP is expressed use ubiquitously expressed promoters. For example, Ikawa et al. utilize the chicken beta-actin promoter and analyze expression from the promoter in transgenic mice, not embryoid bodies. The presently claimed approach is completely different from that of Ikawa et al., which generated green mice under the control of a constitutive promoter. Moreover, the senior author Ikawa et al., Dr. Masaru Okabe, indicated in an e-mail to colleagues of the Inventor that GFP may not be a suitable marker for use in embryonic stem cells (see Paragraph 5 of the Hescheler Declaration).

The claimed invention relates to a GFP reporter gene construct which is substantially inactive in undifferentiated stem cells. In contrast, Zernicka-Goetz et al. merely discloses the production of chimeric mice expressing GFP in proliferating cells by means of a GFP gene linked to the cdc2 promoter. Zernicka-Goetz et al. does not teach or suggest that the particular combination of a promoter that is substantially inactive in an

undifferentiated stem cell with a reporter gene encoding a fluorescent protein to monitor cell type- and development-specific gene expression.

According to the present invention, the term "a cell-dependent promoter" refers to a promoter which displays its promoter activity only in particular cell types. The term "development-dependent promoter" refers to a promoter which displays its promoter activity only in particular stages of cellular development (see, e.g., page 4, last paragraph to page 5, second paragraph of the specification). Examples for cell-dependent and development-dependent promoters may include those specific for cardiac tissue, such as the human heart-specific alpha-actin promoter and the Nkx-2.5 promoter specific for early cardiomyocytes. According to the present invention, examples for preferred constructs containing a cell- and/or development-dependent promoter include pCX(alpha-act)GFP-Neo (DSM 11633).

Considering the definition of the cell-dependent and development-dependent promoters, these promoters are active only in very selected parts of embryoid bodies while in the rest of the embryoid body the promoter is non-active. With reference to Example 2 of the present specification, the heart-specific alpha-actin promoter is active only in a very limited percentage of the whole cell population of the embryoid bodies.

In contrast, the cdc2 promoter is a proliferation-related promoter which is constitutively active in undifferentiated ES cells. Thus, the cdc2 promoter describes the actual status of a cell but does not fall under the definition of a cell-dependent or development-dependent promoter. For instance, during early stages of development all different cell types do display proliferation which excludes therefore the discrimination between the different cell types. Thus, it appears that all intact cells will express MmGFP under control of the cdc2 promoter. In fact, in the ES cell lines described in Zernicka-Goetz et al., MmGFP is continuously expressed under the control of the cdc2 promoter even after several months in culture (see page 1135, right column, first paragraph). It was only after the ES cells exited the cell cycle and differentiated in culture that MmGFP fluorescence could not be detected in the non-dividing differentiated cells.

In this respect, the Applicant would like to point out that the use of a constitutive promoter, such as the cdc2 promoter for expression of GFP, makes a substantial difference in the expectation to be successful compared to the use of a cell- or tissue-

specific promoter. This is because of the fact that when transfecting stem cells with a GFP reporter gene construct, wherein the reporter gene is constitutively expressed, i.e. immediately after introducing the reporter gene construct into the cells, the transfected cells will necessarily be selected for stable transformants which tolerate the expression of the foreign GFP gene. Accordingly, the authors of Zernicka-Goetz et al. were successful in establishing an ES cell line which continuously expresses MmGFP.

In contrast, when using the cell type- or tissue-specific promoter, the transformed ES cells are confronted with the expression of GFP only at a particular stage of cellular development. Thus, the sort of preselection as possible for ES cell lines continuously expressing GFP is not possible so that the person skilled in the art had no reasonable expectation whether or not the sudden onset of GFP expression may disturb cell viability and/or its further development.

The present invention for the first time shows that ES cells strongly expressing GFP at a particular developmental stage can differentiate into functionally mature contractile cardiomyocytes which have the same basic electro-physiological properties as cardiomyocytes which have differentiated from "normal" D3 cells. It has been known in the art that the differentiation in organisms is crucially dependent on delicate chronological and quantitative interplay of specific sets of transcription factors. However, it was unknown, before the filing date of the present application, whether tissue-specific expression of the fluorescent reporter gene as claimed in the present application may alter and/or disturb the mechanisms driven by the balance of the signals that are crucially important for development. The functional experiments of the present invention have revealed that tissue-specific expression of the reporter gene of the present invention does not affect the mechanisms for differentiation in organisms.

As can be seen, none of the references, alone or in combination, indicate that the processes and compositions of the present invention **would** have been successful. None of the cited references address the issues discussed above. Many problems existed that were recognized in the art and would have led one of skill in the art to use established reporter systems such as beta-galactosidase instead of the more experimental and less sensitive GFP. As noted in Section 4a above, the Examiner has failed to address these

arguments, the Raz et al. reference, or the Okabe e-mail. Accordingly, Applicants request that the obviousness rejection be removed.

CONCLUSION

All grounds of rejection and objection of the Final Office Action of August 25, 2004 having been addressed, reconsideration of the application is respectfully requested. It is respectfully submitted that the invention as claimed fully meets all requirements and that the claims are worthy of allowance. Should the Examiner believe that a telephone interview would aid in the prosecution of this application, Applicant encourages the Examiner to call the undersigned collect at (608) 218-6900.

Dated: 11-24-04

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